TABLE E2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 11-Month Interim Evaluation in the Lifetime Inhalation Study of Talc[a]

	0 mg/m³	6 mg/m³	18 mg/m³
Male			
n	2	3	3
Necropsy body wt	425 ± 10	406 ± 15	395 ± 14
Brain			
Absolute	2.018 ± 0.010	1.616 ± 0.306	2.020 ± 0.012
Relative	4.75 ± 0.13	3.97 ± 0.74	5.13 ± 0.16
Heart			
Absolute	1.161 ± 0.080	1.051 ± 0.063	1.079 ± 0.048
Relative	2.73 ± 0.12	2.58 ± 0.06	2.73 ± 0.09
R. Kidney			
Absolute	1.313 ± 0.008	1.242 ± 0.062	1.216 ± 0.069
Relative	3.09 ± 0.09	3.07 ± 0.26	3.07 ± 0.07
Liver			
Absolute	12.824 ± 0.065	12.454 ± 0.424	12.223 ± 0.618
Relative	30.20 ± 0.86	30.72 ± 1.47	30.92 ± 0.50
Lungs			
Absolute	1.228 ± 0.143	1.152 ± 0.043	1.979 ± 0.077**
Relative	2.90 ± 0.40	2.85 ± 0.18	5.02 ± 0.16**
Female			
n	3	3	3
Necropsy body wt	254 ± 7	249 ± 5	247 ± 10
Brain			
Absolute	1.863 ± 0.003	1.867 ± 0.036	1.845 ± 0.030
Relative	7.36 ± 0.22	7.52 ± 0.18	7.50 ± 0.19
Heart			
Absolute	0.858 ± 0.032	0.796 ± 0.020	0.753 ± 0.063
Relative	3.38 ± 0.06	3.20 ± 0.06	3.05 ± 0.19
R. Kidney			
Absolute	0.830 ± 0.007	0.839 ± 0.002	0.735 ± 0.034*
Relative	3.28 ± 0.11	3.38 ± 0.07	2.99 ± 0.13
Liver			
Absolute	7.878 ± 0.275	7.774 ± 0.130	7.537 ± 0.354
Relative	31.13 ± 1.53	31.30 ± 0.47	30.57 ± 0.50
Lungs			
Absolute	0.959 ± 0.037	1.039 ± 0.034	1.551 ± 0.163**
Relative	3.79 ± 0.20	4.18 ± 0.09	6.27 ± 0.48**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

Table E3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 18-Month Interim Evaluation in the Lifetime Inhalation Study of Talc[a]

	0 mg/m^3	6 mg/m^3	18 mg/m^3
Male			
n	3	3	2
Necropsy body wt	446 ± 14	428 ± 10	430 ± 2
Brain			
Absolute	2.019 ± 0.043	1.965 ± 0.035	2.092 ± 0.004
Relative	4.53 ± 0.10	4.60 ± 0.17	4.86 ± 0.01
Heart			
Absolute	1.077 ± 0.065	1.027 ± 0.030	1.131 ± 0.103
Relative	2.41 ± 0.09	2.40 ± 0.07	2.63 ± 0.23
R. Kidney			
Absolute	1.913 ± 0.599	1.328 ± 0.063	1.317 ± 0.023
Relative	4.27 ± 1.31	3.10 ± 0.12	3.06 ± 0.06
Liver			
Absolute	14.329 ± 1.434	13.866 ± 0.882	12.520 ± 0.189
Relative	32.10 ± 3.01	32.38 ± 1.68	29.10 ± 0.56
Lungs			
Absolute	1.691 ± 0.100	1.852 ± 0.058	3.169 ± 0.121**
Relative	3.78 ± 0.13	4.34 ± 0.21	7.36 ± 0.25**
Female			
n	3	3	3
Necropsy body wt	305 ± 5	275 ± 4**	280 ± 4*
Brain			
Absolute	1.840 ± 0.028	1.827 ± 0.045	1.847 ± 0.013
Relative	6.04 ± 0.17	6.63 ± 0.11*	6.61 ± 0.13*
Heart			
Absolute	0.772 ± 0.015	0.706 ± 0.010*	0.765 ± 0.011
Relative	2.53 ± 0.08	2.56 ± 0.03	2.74 ± 0.01*
R. Kidney			
Absolute	0.929 ± 0.023	0.902 ± 0.038	0.955 ± 0.047
Relative	3.05 ± 0.12	3.28 ± 0.17	3.41 ± 0.13
Liver			
Absolute	8.750 ± 0.223	8.540 ± 0.648	8.904 ± 0.596
Relative	28.71 ± 0.35	31.03 ± 2.47	31.84 ± 1.94
Lungs			
Absolute	1.130 ± 0.026	1.395 ± 0.046**	2.600 ± 0.030**
Relative	3.71 ± 0.12	5.07 ± 0.11**	9.31 ± 0.18**

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

TABLE E4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 24-Month Interim Evaluation in the Lifetime Inhalation Study of Talc[a]

	0 mg/m³	6 mg/m³	18 mg/m³
Male			
n	3	6	2
Necropsy body wt	406 ± 29	422 ± 12	392 ± 30
Brain			
Absolute	2.068 ± 0.015	2.023 ± 0.025	1.989 ± 0.008
Relative	5.15 ± 0.42	4.81 ± 0.11	5.10 ± 0.37
Heart			
Absolute	1.065 ± 0.022	1.126 ± 0.044	0.993 ± 0.026
Relative	2.66 ± 0.25	2.69 ± 0.18	2.54 ± 0.13
R. Kidney			
Absolute	1.720 ± 0.138	1.577 ± 0.048	1.649 ± 0.068
Relative	4.25 ± 0.32	3.76 ± 0.19	4.24 ± 0.50
Liver			
Absolute	15.298 ± 0.187	14.924 ± 0.480	14.344 ± 1.253
Relative	38.11 ± 3.23	35.55 ± 1.80	37.05 ± 6.03
Lungs			
Absolute	1.766 ± 0.177	2.150 ± 0.230	2.473 ± 0.674
Relative	4.40 ± 0.55	5.18 ± 0.69	6.48 ± 2.21
Female			
n	5	9	3
Necropsy body wt	296 ± 17	296 ± 10	262 ± 25
Brain			
Absolute	1.821 ± 0.023	1.826 ± 0.011	1.865 ± 0.012
Relative	6.24 ± 0.42	6.24 ± 0.21	7.23 ± 0.63
Heart			
Absolute	0.826 ± 0.014	0.826 ± 0.032	0.824 ± 0.045
Relative	2.83 ± 0.19	2.81 ± 0.10	3.16 ± 0.13
R. Kidney			
Absolute	1.118 ± 0.055	1.137 ± 0.044	1.021 ± 0.022
Relative	3.82 ± 0.26	3.85 ± 0.10	3.97 ± 0.44
Liver			
Absolute	11.218 ± 0.527	12.127 ± 0.672	9.966 ± 0.246
Relative	38.38 ± 2.74	41.16 ± 2.12	38.84 ± 4.59
Lungs			
Absolute	1.014 ± 0.104	1.447 ± 0.219	3.261 ± 0.115**
Relative	3.40 ± 0.23	4.88 ± 0.67	12.73 ± 1.62**

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

TABLE E5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the Termination of the Lifetime Inhalation Study of Talc[a]

	0 mg/m³	6 mg/m³	18 mg/m³
Male			
n	8	12	13
Necropsy body wt	379 ± 17	397 ± 6	326 ± 12**
Brain			
Absolute	2.030 ± 0.016	2.041 ± 0.015	2.014 ± 0.019
Relative	5.45 ± 0.28	5.16 ± 0.09	6.29 ± 0.25*
Heart			
Absolute	1.385 ± 0.104	1.288 ± 0.041	1.302 ± 0.064
Relative	3.68 ± 0.26	3.26 ± 0.13	4.05 ± 0.22
R. Kidney			
Absolute	1.899 ± 0.151	1.847 ± 0.125	1.737 ± 0.101
Relative	5.09 ± 0.49	4.69 ± 0.37	5.39 ± 0.35
Liver			
Absolute	15.501 ± 0.861	16.562 ± 0.540	14.055 ± 0.936
Relative	41.03 ± 1.67	41.92 ± 1.73	42.85 ± 1.76
Lungs			
Absolute	2.154 ± 0.124	2.509 ± 0.068	4.026 ± 0.196**
Relative	5.76 ± 0.38	6.34 ± 0.21	12.65 ± 0.85**
Female			
n	12	13	9
Necropsy body wt	260 ± 14	247 ± 14	231 ± 9
Brain			
Absolute	1.975 ± 0.122	1.860 ± 0.020	1.847 ± 0.028
Relative	8.03 ± 0.95	7.89 ± 0.51	8.06 ± 0.27
Heart			
Absolute	1.020 ± 0.039	1.006 ± 0.026	1.047 ± 0.027
Relative	4.03 ± 0.24	4.33 ± 0.39	4.58 ± 0.20
R. Kidney			
Absolute	1.313 ± 0.047	1.235 ± 0.049	1.281 ± 0.079
Relative	5.21 ± 0.34	5.22 ± 0.36	5.66 ± 0.55
Liver			
Absolute	12.005 ± 0.660	12.567 ± 0.903	12.313 ± 0.642
Relative	46.35 ± 1.68	51.25 ± 2.90	53.69 ± 3.72
Lungs			
Absolute	1.575 ± 0.109	2.673 ± 0.362**	4.050 ± 0.228**
Relative	6.11 ± 0.35	11.77 ± 2.10*	17.83 ± 1.43**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

TABLE E6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 6-Month Interim Evaluation in the 2-Year Inhalation Study of Talc[a]

	0 mg/m³	6 mg/m³	18 mg/m³
Male			
n	4	4	4
Necropsy body wt	31.3 ± 0.9	31.1 ± 0.9	32.1 ± 0.6
Brain			
Absolute	0.431 ± 0.028	0.458 ± 0.006	0.469 ± 0.008
Relative	13.81 ± 0.90	14.74 ± 0.23	14.60 ± 0.38
Heart			
Absolute	0.159 ± 0.003	0.165 ± 0.008	0.157 ± 0.011
Relative	5.10 ± 0.07	5.31 ± 0.33	4.88 ± 0.25
R. Kidney			
Absolute	0.303 ± 0.022	0.297 ± 0.018	0.292 ± 0.011
Relative	9.66 ± 0.40	9.58 ± 0.70	9.10 ± 0.33
Liver			
Absolute	1.737 ± 0.079	1.792 ± 0.066	1.731 ± 0.060
Relative	55.51 ± 1.06	57.75 ± 2.77	53.84 ± 1.19
Lungs			
Absolute	0.165 ± 0.008	0.149 ± 0.010	0.173 ± 0.017
Relative	5.29 ± 0.35	4.78 ± 0.27	5.35 ± 0.44
Female			
n	4	4	4
Necropsy body wt	27.1 ± 0.9	27.2 ± 1.7	29.5 ± 1.4
Brain			
Absolute	0.474 ± 0.007	0.482 ± 0.008	0.474 ± 0.019
Relative	17.52 ± 0.36	17.85 ± 0.81	16.10 ± 0.67
Heart			
Absolute	0.142 ± 0.004	0.133 ± 0.005	0.145 ± 0.006
Relative	5.27 ± 0.30	4.92 ± 0.19	4.92 ± 0.15
R. Kidney			
Absolute	0.201 ± 0.011	0.203 ± 0.004	0.217 ± 0.008
Relative	7.40 ± 0.20	7.53 ± 0.34	7.37 ± 0.13
Liver			
Absolute	1.541 ± 0.099	1.640 ± 0.138	1.628 ± 0.033
Relative	56.86 ± 2.92	60.01 ± 1.74	55.38 ± 1.91
Lungs			
Absolute	0.190 ± 0.019	0.164 ± 0.011	0.178 ± 0.011
Relative	7.11 ± 0.96	6.03 ± 0.28	6.04 ± 0.26

[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

TABLE E7
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 12-Month Interim Evaluation in the 2-Year Inhalation Study of Talc[a]

	0 mg/m³	6 mg/m³	18 mg/m³
Male			
n	4	4	4
Necropsy body wt	34.6 ± 1.7	37.2 ± 0.3	33.1 ± 1.3
Brain			
Absolute	0.478 ± 0.020	0.475 ± 0.009	0.475 ± 0.009
Relative	13.87 ± 0.31	12.76 ± 0.16	14.39 ± 0.38
Heart			
Absolute	0.196 ± 0.023	0.195 ± 0.005	0.205 ± 0.023
Relative	5.62 ± 0.37	5.23 ± 0.10	6.21 ± 0.69
R. Kidney			
Absolute	0.334 ± 0.007	0.339 ± 0.020	0.311 ± 0.027
Relative	9.71 ± 0.28	9.12 ± 0.52	9.41 ± 0.86
Liver			
Absolute	1.612 ± 0.052	1.886 ± 0.124	1.928 ± 0.240
Relative	46.77 ± 0.79	50.72 ± 3.25	58.55 ± 8.01
Lungs			
Absolute	0.157 ± 0.009	0.216 ± 0.018	0.243 ± 0.032*
Relative	4.54 ± 0.17	5.80 ± 0.46	7.30 ± 0.72**
Female			
n	3	4	4
Necropsy body wt	32.1 ± 2.4	33.3 ± 1.3	28.7 ± 1.2
Brain			
Absolute	0.478 ± 0.006	0.488 ± 0.005	0.491 ± 0.008
Relative	15.04 ± 1.16	14.74 ± 0.70	17.16 ± 0.55
Heart			
Absolute	0.151 ± 0.004	0.162 ± 0.008	0.190 ± 0.010*
Relative	4.72 ± 0.23	4.91 ± 0.42	6.64 ± 0.47*
R. Kidney			
Absolute	0.225 ± 0.010	0.231 ± 0.008	0.230 ± 0.011
Relative	7.03 ± 0.22	6.97 ± 0.40	8.01 ± 0.10
Liver			
Absolute	1.470 ± 0.105	1.796 ± 0.036*	1.477 ± 0.093
Relative	46.04 ± 3.71	54.20 ± 2.55	51.40 ± 2.48
Lungs			
Absolute	0.151 ± 0.019	0.191 ± 0.014	0.207 ± 0.015*
Relative	4.68 ± 0.23	5.78 ± 0.61	7.19 ± 0.24**

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)

TABLE E8
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 18-Month Interim Evaluation in the 2-Year Inhalation Study of Talc[a]

	0 mg/m³	6 mg/m³	18 mg/m³
Male			
n	4	4	4
Necropsy body wt	33.1 ± 3.0	37.5 ± 2.1	35.4 ± 1.7
Brain			
Absolute	0.467 ± 0.007	0.470 ± 0.009	0.496 ± 0.014
Relative	14.51 ± 1.44	12.63 ± 0.58	14.10 ± 0.76
Heart			
Absolute	0.193 ± 0.017	0.186 ± 0.011	0.203 ± 0.006
Relative	6.18 ± 1.29	5.00 ± 0.35	5.77 ± 0.22
R. Kidney			
Absolute	0.342 ± 0.007	0.361 ± 0.021	0.350 ± 0.009
Relative	10.66 ± 1.23	9.66 ± 0.47	9.91 ± 0.22
Liver			
Absolute	1.844 ± 0.228	1.796 ± 0.080	1.748 ± 0.113
Relative	57.08 ± 7.95	48.07 ± 1.26	49.28 ± 1.45
Lungs			
Absolute	0.229 ± 0.034	0.238 ± 0.013	0.345 ± 0.032*
Relative	7.45 ± 2.01	6.42 ± 0.57	9.79 ± 0.91
Female			
n	4	4	4
Necropsy body wt	32.1 ± 1.2	31.9 ± 1.6	27.6 ± 1.0*
Brain			
Absolute	0.483 ± 0.007	0.467 ± 0.019	0.501 ± 0.038
Relative	15.10 ± 0.59	14.73 ± 0.90	18.33 ± 1.91
Heart			
Absolute	0.155 ± 0.008	0.154 ± 0.011	0.164 ± 0.010
Relative	4.85 ± 0.28	4.87 ± 0.47	5.96 ± 0.48
R. Kidney			
Absolute	0.238 ± 0.009	0.233 ± 0.011	0.228 ± 0.007
Relative	7.41 ± 0.28	7.35 ± 0.45	8.32 ± 0.55
Liver			
Absolute	1.446 ± 0.056	1.592 ± 0.034	1.318 ± 0.055[b]
Relative	45.10 ± 1.35	50.17 ± 2.02	48.69 ± 0.30[b]
Lungs			
Absolute	0.223 ± 0.008	0.242 ± 0.018	0.299 ± 0.018**
Relative	6.96 ± 0.07	7.65 ± 0.73	10.90 ± 0.87**

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
a Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)
b n=3

TABLE E9
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the Termination of 2-Year Inhalation Study of Talc[a]

	0 mg/m³	6 mg/m³	18 mg/m³
Male			
n	30	28	32
Necropsy body wt	33.4 ± 0.5	32.1 ± 0.8	31.2 ± 0.4**
Brain			
Absolute	0.461 ± 0.004	0.458 ± 0.004	0.460 ± 0.005
Relative	13.90 ± 0.22	14.50 ± 0.34	14.78 ± 0.19*
Heart			
Absolute	0.183 ± 0.003	0.181 ± 0.004	0.183 ± 0.005
Relative	5.52 ± 0.12	5.68 ± 0.10	5.88 ± 0.15
R. Kidney			
Absolute	0.361 ± 0.010	0.362 ± 0.010	0.354 ± 0.006
Relative	10.85 ± 0.27	11.28 ± 0.16	11.34 ± 0.18
Liver			
Absolute	1.845 ± 0.064	1.733 ± 0.073[b]	1.535 ± 0.033**[c]
Relative	55.64 ± 2.21	53.14 ± 1.72[b]	49.27 ± 1.03*[c]
Lungs			
Absolute	0.252 ± 0.008[c]	0.258 ± 0.009[b]	0.408 ± 0.011**
Relative	7.47 ± 0.25[c]	8.01 ± 0.24[b]	13.08 ± 0.33**
Female			
n	30	23	25
Necropsy body wt	31.4 ± 0.6	31.7 ± 0.7	30.7 ± 0.5
Brain			
Absolute	0.484 ± 0.004	0.469 ± 0.006	0.477 ± 0.003
Relative	15.53 ± 0.26	14.93 ± 0.28	15.59 ± 0.20
Heart			
Absolute	0.164 ± 0.005	0.190 ± 0.009**	0.163 ± 0.003
Relative	5.24 ± 0.15	6.02 ± 0.28**	5.32 ± 0.09
R. Kidney			
Absolute	0.251 ± 0.007[d]	0.265 ± 0.010	0.257 ± 0.007[e]
Relative	8.03 ± 0.17[d]	8.38 ± 0.27	8.37 ± 0.14[e]
Liver			
Absolute	1.816 ± 0.089	1.770 ± 0.107[f]	1.761 ± 0.083[e]
Relative	57.41 ± 2.25	55.45 ± 3.13[f]	56.94 ± 1.93[e]
Lungs			
Absolute	0.276 ± 0.014	0.293 ± 0.012	0.410 ± 0.010**
Relative	8.80 ± 0.42	9.28 ± 0.34	13.39 ± 0.28**

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error)
[b] n=27
[c] n=28
[d] n=29
[e] n=24
[f] n=22

APPENDIX F
LUNG BURDEN, PULMONARY FUNCTION, AND LUNG BIOCHEMISTRY IN RATS

METHODS

Lung Burden

Lung talc burden was measured to determine the relationship between the exposure concentration and the amount of talc deposited and retained within the pulmonary region of the respiratory tract. The method used for analyzing for talc in lungs has been published (Hanson *et al.*, 1985). Lung burdens were determined on 3 male and 3 female rats from each exposure group sacrificed at 27, 47, 79, and 105 weeks after the start of exposure. The analysis was based on determination of acid insoluble magnesium in the lung. MRI reported that the value for the magnesium was 19.33% for batch 02 and 19.47% for batch 03. These values and the results of the analysis at LITRI were close to the theoretical value of magnesium for talc (19.22%). Since rats sacrificed at 27, 47, and 79 weeks had been exposed to only batch 02 of talc, 19.33% magnesium was used to calculate the quantity of talc for these rats. Because batch 03 was used for the last 4 months of exposure and lung burdens of rats after 105 weeks of exposure to talc would be expected to contain substantial amounts of batch 03 talc, 19.47% magnesium was used to calculate the quantity of talc deposited in the lungs of these rats.

All operations in conjunction with tissue analysis for talc were done while wearing talc-free gloves. Left lung lobes were weighed at necropsy and stored frozen (-20° C) until used. Lungs were homogenized using water and the proteins were precipitated with 70% perchloric acid. The individual samples were filtered and washed with 5% trichloroacetic acid (TCA) to remove perchlorates. Washing continued until magnesium levels in the wash were within 10% of levels in the TCA solution ($\leq$ 0.03 ppm magnesium). Filters and tissue residues were placed in 15 mL porcelain crucibles, dried slowly (200° C), and then ashed at 600° C for 1 hour. Ashed samples were transferred to Teflon beakers using 2 mL HCl and evaporated to dryness. Samples were then digested in hydrofluoric acid (HF), and the HF evaporated. Additional HF was added and reevaporated. Sulfuric acid was added to remove trace HF, and samples were then diluted with distilled water and analyzed for magnesium by atomic absorbance (Perkin Elmer, Model 306, Atomic Absorption Spectrophotometer) with a magnesium hollow cathode lamp and an air acetylene flame (Hanson *et al.*, 1985).

Pulmonary Function

Ten male and 10 female rats from each exposure level were assigned for respiratory function analyses. Respiratory function was measured at 6 months (27 weeks), 10 months (43 weeks), and 18 months (79 weeks). At 24 months (103 to 104 weeks) of exposure, respiratory function was performed on all surviving rats not assigned to the lifetime study. Respiratory function was measured by noninvasive techniques, using methods previously published (Harkema *et al.*, 1982).

Tests were conducted using a 1.4 L combination flow and pressure plethysmograph. Flows were measured by measuring differential pressures across a wire screen pneumotachograph in the plethysmograph wall. Volumes were obtained by integration (Model 6, Pulmonary Mechanics Analyzer, Buxco Electronics, Sharon, CT). In the pressure mode, used only for measuring functional residual capacity, the pneumotachograph hole was sealed and volume changes were measured as pressure changes. The plethysmograph was maintained at approximately 37° C by a resistance element. Transpulmonary pressure was measured using transducers connected to the external airway and a liquid-filled, 2.2 mm O.D. esophageal catheter.

A positive-negative pressure respirator system was used to induce quasistatic and forced respiratory movements, simulating the movements performed voluntarily by man. Reservoirs maintained at +40 and -50 cm H_2O were connected to the airway by solenoid valves. Inspiratory and quasistatic expiratory flow rates were limited by calibrated needle valves to 5 and 3 mL/sec, respectively. Inspirations were stopped automatically at a transpulmonary pressure of 30 cm H_2O, defining the lung volume at that distending pressure as total lung capacity (TLC). Forced inhalations were induced from TLC by opening the airway to the negative pressure reservoir via a rapidly opening valve having a 9.5 mm I.D., with no intentional flow restriction between the valve and the reservoir.

The rats were anesthetized with halothane and intubated orally with a tracheal catheter 5.5 cm long × 1.8 mm I.D., fabricated from a 14-gauge intravenous catheter as previously described (Mauderly, 1977).

The breathing port in the plethysmograph wall was a luer fitting drilled to 2.5 mm I.D. The frequency response of the plethysmograph-respirator-tracheal catheter system has been tested and found adequate for forced expiratory events in rats. No phase lag among flow, pressure and volume signals has been found in the frequency range of spontaneous breathing.

Rats were anesthetized, intubated and placed prone in the plethysmograph. The esophageal catheter was adjusted to maximize the recorded transpulmonary pressure signal. Anesthetic depth was adjusted to yield a respiratory frequency of 50 to 60 per minute. Respiratory frequency, tidal volume, minute volume, dynamic lung compliance, and total pulmonary resistance were recorded during spontaneous respiration, time-averaged by a data logger and displayed on a teletype terminal.

Prior to each subsequent measurement procedure, the rat's lung was manually inflated with a syringe to induce apnea. A quasistatic deflation from TLC to residual volume allowed measurement of vital capacity and the quasistatic expiratory pressure-volume curve. Quasistatic lung chord compliance was measured as the slope of the curve over the chord between the apneic lung volume and the volume at +10 cm H_2O pressure. Maximum quasistatic compliance was measured as the steepest slope of the pressure-volume curve over any 2 cm H_2O pressure interval. Functional residual capacity was measured by the barometric method (Dubois *et al.*, 1956) from recordings of lung volume and airway pressure changes as the rat resumed breathing against a blocked airway. From these measurements, all subdivisions of lung volume were calculated including residual volume.

Alveolar-capillary gas exchange was evaluated by a single-breath, CO diffusing capacity test (Ogilvie *et al.*, 1957). The lungs were inflated with a gas mixture containing CO and Ne in air to 20 cm H_2O transpulmonary pressure. After 6 seconds, one-half of the gas was withdrawn and the remaining gas collected for analysis by gas chromatography. The lung volume when inflated with the mixture was measured by neon dilution.

A forced inhalation was performed as described above, and the maneuver analyzed by a microprocessor in the data logger (Model D-12, Buxco). Data included forced vital capacity (FVC), the percentage of FVC exhaled in 0.1 second, flow rates at peak flow, and at 50%, 25%, and 10% of FVC.

A single-breath nitrogen washout was performed by recording volume and nitrogen concentration of expirate during a slow deflation after an inflation to TLC with oxygen. The slope of phase III ("alveolar plateau") of the washout curve was calculated to assess the uniformity of intrapulmonary gas distribution.

Lung Biochemistry

All surviving rats from each exposure group (the 3 males and 3 females originally assigned for lung burden/histology and the 10 males and 10 females from physiology/biochemistry) were sacrificed after 105 weeks of exposure.

The rats were anesthetized with halothane and sacrificed by exsanguination from the abdominal aorta or renal artery. The heart and lung block was removed, the right apical, right cardiac, and right intermediate portions of each rat lung were given endobronchial saline lavage (6 mL total volume in three, 2.0 mL washes of saline), and the bronchoalveolar lavage (BAL) fluid was centrifuged at 300 × G to separate the cells from the supernatant fluid.

Airway Fluid Enzymes and Cytology Measurements

In this study, BAL fluid was analyzed to determine the degree of:

1) Cell injury as indicated by concentration of lactate dehydrogenase (LDHP)
2) Chronic inflammatory response as indicated by presence of increased numbers of polymorphonuclear leukocytes (PMN) and pulmonary alveolar macrophages (AM) as well as increased protein and alkaline phosphatase activity

3) Lysosomal activation as indicated by ß-glucuronidase and acid proteinase activity. Elevated enzyme activities have been observed in BAL fluid from rodents exposed to particles. These enzymes may be associated with the breakdown of necrotic tissues.
4) Response to oxidant injury as indicated by increased glutathione reductase activity.

The supernatant fluid was analyzed by spectrophotometric, kinetic, and enzymatic analyses for the activities of ß-glucuronidase, LDHP, glucose-6-phosphate dehydrogenase, alkaline phosphatase, glutathione reductase, and glutathione peroxidase. Acid proteinase was measured by the release of radiolabeled globin peptides from the trichloroacetic acid-precipitable protein substrate, and total protein was analyzed colorimetrically (Henderson *et al.*, 1985).

Numbers of total nucleated cells recovered in lavage fluid were determined using a cell counter (Coulter Electronic, Hileah, FL) or a hemocytometer. Cytocentrifuge preparations of resuspended cells were made, stained with Wright's stain (Diff-Quick, Curtin Matheson Scientific, Denver, CO) and the differential cell count determined.

Alveolar macrophages (AM) were recovered from BAL fluid of the same rats as described above. The cells (1×10^6) were suspended in Roswell Park Memorial Institute (RPMI) 1640 culture medium and pelleted by centrifugation and the supernatant RPMI removed. Cells were resuspended in 1 mL of a 1% suspension of IgG antibody-sensitized sheep red blood cells (SRBC) in RPMI 1640. The antibody-sensitized SRBC were made as previously described (Harmsen and Jeska, 1980). The subagglutinating titer of heat-inactivated rabbit anti-SRBC serum was used to sensitize the SRBC. The AM and SRBC suspensions were incubated at 37° C for 1 hour in a humidified atmosphere of 5% CO_2 in air. The AM and SRBC were sedimented by centrifugation and the supernatant discarded. Unphagocytized SRBC were removed by lysing the RBC with water for 30 seconds. Lysing of unphagocytized SRBC was stopped by the addition of an equal volume of saline and cytocentrifuge preparations were made. The slides were stained with Wright's stain (Diff-Quik, American Scientific Products, McGaw Park, IL) and the percent of AM phagocytizing SRBC was determined by light microscopy. Three fields of 100 cells per preparation were counted. Viability was determined by trypan blue exclusion.

Lung Tissue Collagen and Proteinase

In this study, rats sacrificed at 105 weeks of talc exposure were used for collagen metabolism, protein synthesis, and proteinase activity measurements. Tissue and BAL fluid from single rats were used for analyses.

To estimate collagen and protein synthesis, ^{14}C-proline (0.1 μCi/g body weight) was injected intraperitoneally approximately 2 to 3 hours prior to sacrifice. Lung lobes to be analyzed for collagen were frozen in liquid nitrogen and pulverized. The pulverized lungs were extracted overnight in 0.5M acetic acid at 4° C, and centrifuged to separate the insoluble material from the supernatant fluid. The supernatant fluid was separated into high and low molecular weight fractions using Amicon Cones with a size cutoff of approximately 50 kDa.

All samples for collagen analyses from lung and lavage supernatant fluid were hydrolyzed for approximately 18 hours in 6N HCl at 110° C to convert proteins to their individual amino acids, were evaporated to dryness to remove the HCl, and were resuspended in 0.001 N HCl prior to analysis.

Collagen quantity was measured and multiplied by 7.46 to convert BAL or lung tissue hydroxyproline content to BAL or lung tissue collagen content, taking into account that collagen is approximately 13% hydroxyproline by weight (Neuman and Logan, 1950).

Radioactive proline and hydroxyproline were quantitated in the low molecular weight supernatant fluid fraction and in a sample containing both the high molecular weight supernatant fluid fraction and the acetic acid insoluble fraction. Following this, the radioactive proline and hydroxyproline quantities were

used to calculate the noncollagenous protein synthesis, the collagen production, and the intracellular collagen degradation.

Noncollagenous protein synthesis was measured as the total radioactive proline incorporation into lung tissue minus the incorporation into lung tissue which was related to collagen synthesis. The radioactive proline in collagen was assumed to be equal to the radioactive hydroxyproline, thus, incorporation into collagen was calculated as twice the radioactive hydroxyproline. Collagen production (% of newly synthesized protein that was collagen) was calculated as the percentage of the total incorporation of proline into all proteins constituted by collagen, and adjusted for the 5.4-fold difference in the content of total amino acids (proline and hydroxyproline) between collagen and noncollagenous protein (Pickrell *et al.*, 1987). Intracellular collagen degradation (as a percent of newly synthesized collagen) was calculated as the percentage of total radioactive hydroxyproline in collagen constituted by low molecular weight radioactive hydroxyproline-containing peptides.

Lung tissue proteinase activity was measured as the release of ^{14}C-leucine from prelabeled globin at pH 4.2 and 7.5 (Gregory and Pickrell, 1982; Harkema *et al.*, 1984; Pickrell *et al.*, 1987). Acid proteinase activity was inhibited by leupeptin to indicate either neutrophil and macrophage cathepsin B (inhibited) or macrophage cathepsin D (not inhibited)-like activity. Neutral proteinase activity was inhibited by 1,10-phenanthroline to indicate either macrophage elastase (inhibited) or neutrophil elastase-cathepsin G (not inhibited)-like activity.

Table F1
Number of Rats Evaluated for Lung Talc Burden, Pulmonary Function, and Lung Biochemistry

	Male			Female		
	0 mg/m^3	6 mg/m^3	18 mg/m^3	0 mg/m^3	6 mg/m^3	18 mg/m^3
Lung Burden						
6-Month Interim	–[a]	3	3	–	3	3
11-Month Interim	–	3	3	–	3	3
18-Month Interim	–	3	3	–	2	3
24-Month Interim	–	6	9	–	2	3
Pulmonary Function						
6-Month Interim	9	10	10	10	10	10
11-Month Interim	9	10	10	10	10	10
18-Month Interim	9	10	10	9	9	9
24-Month Interim	3	6	3	6	9	3
Lung Biochemistry						
24-Month Interim	3	6	2	5	9	3

[a] Lung burden not measured in 0 mg/m^3 rats.

TABLE F2
Lung Talc Burden (Normalized to Control Lung Weight) of Rats[a]

	6 months	12 months	18 months	24 months
Male				
0 mg/m³	–[b]	–	–	–
6 mg/m³	2.63 ± 0.24	4.38 ± 0.59	7.31 ± 0.71	10.45 ± 1.26
18 mg/m³	10.83 ± 0.23	20.96 ± 2.04	27.57 ± 0.91	24.15 ± 3.41
Female				
0 mg/m³	–	–	–	–
6 mg/m³	2.43 ± 0.19	4.71 ± 0.26	7.66 ± 0.34	9.10 ± 0.88
18 mg/m³	8.34 ± 0.12	14.16 ± 3.36	24.33 ± 0.63	29.40 ± 2.40

[a] Units are presented as mg talc/g control lung.
[b] No measurements taken

TABLE F3
Lung Talc Burden (Normalized to Exposure Concentration) of Rats[a]

	Male		Female	
	6 mg/m³	18 mg/m³	6 mg/m³	18 mg/m³
6-Month Interim	0.439 ± 0.040	0.602 ± 0.013*	0.406 ± 0.032	0.464 ± 0.007*
12-Month Interim	0.731 ± 0.098	1.165 ± 0.113*	0.785 ± 0.043	0.787 ± 0.187
18-Month Interim	1.22 ± 0.12	1.53 ± 0.05	1.28 ± 0.06	1.35 ± 0.04
24-Month Interim	1.74 ± 0.21	1.34 ± 0.19	1.52 ± 0.15	1.63 ± 0.13

* Significantly different ($P \leq 0.05$) from the 6 mg/m³ group by Dunn's or Shirley's test
[a] Units are presented as mg talc/g control lung/mg/m³

TABLE F4
Bronchoalveolar Lavage Fluid Enzymes of Rats at the 24-Month Interim Evaluation

	0 mg/m³	6 mg/m³	18 mg/m³
Male			
ß-Glucuronidase[a]	1.09 ± 0.40	18.86 ± 3.20*	89.24 ± 14.24**
Lactate dehydrogenase	1,634 ± 545	3,193 ± 606	8,262 ± 380*
Alkaline phosphatase	364.7 ± 147	572.8 ± 86.8	1,604.7 ± 143*
Glutathione reductase	103.03 ± 16.43	99.35 ± 19.79	110.99 ± 51.27
Total protein[b]	1.78 ± 0.40	3.12 ± 0.64	5.79 ± 0.55*
Female			
ß-Glucuronidase	3.33 ± 0.97	41.05 ± 4.39**	154.16 ± 17.21**
Lactate dehydrogenase	1,655 ± 266	3,906 ± 444*	14E3 ± 1E3**
Alkaline phosphatase	427.8 ± 30.9	853.6 ± 79.7**	2,504.7 ± 221**
Glutathione reductase	100.6 ± 1.7	135.2 ± 22.4	460.0 ± 44.8*
Total protein	1.20 ± 0.22	4.30 ± 0.36**	12.96 ± 0.28**

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Units presented as mIU/g control lung
[b] Units presented as mg/g control lung

TABLE F5
Bronchoalveolar Lavage Fluid Cell Populations of Rats at the 24-Month Interim Evaluation

	0 mg/m³	6 mg/m³	18 mg/m³
Male			
Polymorphonuclear cells[a]	0.333 ± 0.167	24.417 ± 2.557*	32.500 ± 3.000*
Lymphocytes	0.000 ± 0.000	0.500 ± 0.258	0.500 ± 0.500
Macrophages	93.67 ± 3.72	70.25 ± 2.53*	62.75 ± 1.75*
Epithelial cells	6.00 ± 3.61	4.83 ± 1.41	4.25 ± 1.75
Female			
Polymophonuclear cells	0.625 ± 0.315	25.778 ± 2.673**	37.000 ± 1.528**
Lymphocytes	0.000 ± 0.000	0.722 ± 0.188*	1.333 ± 0.667*
Macrophages	91.38 ± 1.75	71.22 ± 2.95**	57.33 ± 4.67**
Epithelial cells	8.00 ± 2.01	2.28 ± 0.50*	4.33 ± 2.60

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Units presented as percent of total cells

TABLE F6
Viability and Phagocytic Activity of Macrophages in Bronchoalveolar Fluid of Rats at the 24-Month Interim Evaluation

	0 mg/m³	6 mg/m³	18 mg/m³
Male			
Viability[a]	63.67 ± 5.91	66.73 ± 1.59	57.70 ± 5.00
Phagocytic activity[b]	83.13 ± 4.54	63.12 ± 8.14	65.30[c]
Female			
Viability	82.65 ± 9.65	74.64 ± 3.24	61.00 ± 4.42
Phagocytic activity	75.60 ± 5.14	66.51 ± 8.09	70.15 ± 2.85

a Units are presented as percent viable cells.
b Units are presented as percent cells phagocytizing sheep erythrocytes.
c n=1; no statistic calculated

TABLE F7
Lung Collagen Metabolism and Protein Synthesis in Rats at the 24-Month Interim Evaluation

	0 mg/m³	6 mg/m³	18 mg/m³
Male			
Lavage fluid collagenous peptides[a]	39.79 ± 5.07	46.99 ± 6.51	79.21 ± 13.73
Total lung collagen[b]	13.87 ± 0.60	15.98 ± 0.39*	18.88 ± 3.35*
Collagen production[c]	1.58 ± 0.17	1.60 ± 0.17	1.63 ± 0.22
Collagen degradation[d]	31.67 ± 1.72	27.74 ± 1.42	9.18 ± 2.38*
Non-collagenous protein synthesis[e]	142.1 ± 14.5	199.8 ± 22.1*	312.2 ± 10.6**
Female			
Lavage fluid collagenous peptides	78.27 ± 11.64	115.36 ± 8.61*	174.71 ± 13.56**
Total lung collagen	14.32 ± 0.66	19.95 ± 1.58*	36.47 ± 3.39**
Collagen production	0.982 ± 0.185	1.804 ± 0.144*	2.264 ± 0.347**
Collagen degradation	14.41 ± 2.44	21.59 ± 4.99	9.38 ± 1.63
Non-collagenous protein synthesis	173.9 ± 34.5	325.8 ± 90.9	554.3 ± 107*

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
a Units are presented as µg/g control lung.
b Units are presented as mg/g control lung.
c Units are presented as percent new protein.
d Units are presented as percent new collagen.
e Units are presented as dpm x 10^3/g control lung.

TABLE F8
Proteinase Activity in Lavage Fluid and Lung Homogenate Supernatant Fluid of Rats at the 24-Month Interim Evaluation[a]

	0 mg/m³	6 mg/m³	18 mg/m³
Male			
Lavage Fluid			
Acid Proteinase	0.994 ± 0.329	1.866 ± 0.174	4.307 ± 0.218*
Cathepsin D	0.147 ± 0.147	0.599 ± 0.150	2.420 ± 0.147**
Cathepsin B	0.924 ± 0.415	1.267 ± 0.094	1.887 ± 0.365
Homogenate Supernatant Fluid			
Acid Proteinase	10.92 ± 0.64	17.51 ± 0.90*	25.13 ± 1.50**
Cathepsin D	8.53 ± 0.91	14.04 ± 0.62*	21.03 ± 1.56**
Cathepsin B	2.39 ± 0.41	3.48 ± 0.37	4.10 ± 0.06*
Neutral Proteinase	0.715 ± 0.168	2.417 ± 0.304*	4.505[b]
PMN Elastase Cathepsin G	0.490 ± 0.218	1.936 ± 0.242*	4.457 ± 0.377**
Macrophage Elastase Collagenase	0.225 ± 0.099	0.482 ± 0.077	0.000[b]
Female			
Lavage Fluid			
Acid Proteinase	1.52 ± 0.12	3.46 ± 0.33*	6.05 ± 0.73**
Cathepsin D	0.015 ± 0.015	1.310 ± 0.292*	4.043 ± 0.578**
Cathepsin B	1.61 ± 0.26	2.15 ± 0.22	2.01 ± 0.17
Homogenate Supernatant Fluid			
Acid Proteinase	14.04 ± 0.95	29.43 ± 1.18**	38.61 ± 1.81**
Cathepsin D	10.05 ± 0.68	22.97 ± 1.07**	30.25 ± 1.60**
Cathepsin B	3.99 ± 0.58	6.46 ± 0.60*	8.37 ± 0.42**
Neutral Proteinase	0.648 ± 0.087	5.040 ± 0.418**	12.293 ± 1.598**
PMN Elastase Cathepsin G	0.785 ± 0.142	4.351 ± 0.261**	10.313 ± 2.694**
Macrophage Elastase Collagenase	0.054 ± 0.037	0.683 ± 0.175*	2.012 ± 1.126*

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
a Units are presented as mg/hour/mg control lung.
b n=1; no statistic calculated